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Determination of Bone Mineral Content in Cadaveric Test Specimens

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ABSTRACT

This paper presents a study designed to determine the best method for presenting the bone mineral content of cadaveric test specimens. A total of 59 bone samples were taken from the humerus, radius, and ulna of 14 female cadaver subjects. Once the samples were cleaned to remove all soft tissue and bone marrow, a dual-energy x-ray absorptiometry scanner was used to determine the bone mineral content and projected area of each sample. The ash weight ratio was calculated from the measured bone mineral content and the dry mass of each sample. The volumetric bone mineral density was found by dividing the bone mineral content by the sample volume as determined by water displacement. A linear regression analysis was performed to compare the ash weigh ratio to the three methods for reporting the bone mineral content: bone mineral content divided by specimen length g/cm, bone mineral content divided by projected area g/cm^2, or bone mineral content divided by specimen volume g/cm^3. For specimens from multiple subjects, the analysis revealed that the ash weight ratio correlates better to the volumetric representation ($R^2 = 0.66$) than the length ($R^2 = 0.21$) or projected area ($R^2 = 0.29$) representations. Additionally, when the subject variance was removed by using multiple specimens from a single subject, the ash weight ratio correlated very well with the volumetric representation for the humerus ($R^2 = 0.84$), radius ($R^2 = 0.95$), and the ulna ($R^2 = 0.94$). Given that all subjects were female with similar anthropometry, the ash weight ratio was found to be independent of age, weight, and height with correlation coefficients of 0.0003, 0.0058, and 0.0018 respectively. A volumetric representation is suggested as the best representation of bone mineralization due to its correlation with ash weight ratio and ability to indicate the level of porosity in the cadaveric specimen

INTRODUCTION

Significant variation in bone strength can exist among individuals due to differences in their age, diet, level of physical activity, and metabolic condition. To account for these differences, scientists commonly use bone mineral content (BMC) as a measure of the bone's strength and fracture risk [1-4]. BMC has been correlated with both the elastic modulus and ultimate strength in the cadaveric specimen [5]. Furthermore, a number of factors directly affect the BMC, such as sex and age. Females present lower values of BMC compared to males, and BMC typically decreases with age [6-9]. Osteoporosis also has been shown to play a primary role in the measured BMC of elderly individuals [10-14]. In addition, menopause has a dramatic affect on BMC with postmenopausal women who do not take a calcium supplement experiencing nearly a 2 % drop in BMC per year [15-19].

The three most commonly used techniques to determine bone mineralization are ashing, quantitative computed tomography (QCT), and dual-energy x-ray absorptiometry (DEXA). Ashing results in an accurate measurement of the mineral content of the sample presented as a percentage of the sample's dry weight. This percentage is referred to as the ash weight ratio (AWR). While ashing has been extensively validated, the process is difficult, time-consuming, and highly sensitive to the ashing temperature. QCT allows for the direct measurement of bone mineral density (BMD) in g/cm^3 [20]. However, QCT is not widely used due to its high cost and radiation burden [21]. Compared to QCT, DEXA has the advantages of lower cost and shorter scanning time which allows for a lower radiation dose [22-25]. A DEXA scan presents the BMC (g) of the entire sample and the projected area (cm^2) of the sample. Together with the sample's length and volume which must be measured independent of the DEXA scanner, the DEXA output may be presented four ways: the BMC alone, the BMC divided by the length of the sample (LBMD), the BMC divided by the projected area (ABMD), and the BMC divided by the sample volume (VBMD) [26].

The purpose of this paper is to determine which method of reporting DEXA results, LBMD, ABMD, or VBMD, best correlates with the AWR, and is the most suitable for use in studies dealing with cadaveric samples. This correlation is needed given the difficulty associated with comparing studies which present BMC in different formats. For example, one study may present the mineral content of a bone segment as LBMD or ABMD, but without knowing the exact specimen length or orientation within the DEXA scanner, it is difficult to compare the results to samples from other studies.

METHODOLOGY

This investigation was limited to cadaveric specimens since the measurement technique used to determine bone volume is highly invasive and could not be done in vivo. Bone samples were taken from the upper extremity due to specimen availability and the fact that DEXA scanners have been validated in previous studies [27-30].

Two studies were performed that utilized bone samples taken from the humerus, radius, and ulna of female cadavers. The first study used bone samples from multiple subjects, while the second study used multiple samples from a single subject. For both studies, pre-test radiographs (frontal and sagittal views) were taken to identify any pre-existing skeletal conditions. If any abnormal bone pathology was observed, the specimen was removed from the test population. The cadavers were obtained through the Virginia State Anatomical Board with permission of the family given to conduct

biomechanics research. All test procedures were approved by the institutional review board at the University of Virginia. Screening for Hepatitis A, B, C, and HIV was conducted with each cadaver prior to acceptance into the research program.

In both studies, the bone samples were cut to approximately 3 cm in length and cleaned to remove all soft tissue and bone marrow from the medullary canal. Hollow polycarbonate cylinders were used to hold the specimens. Four bone samples were placed in each cylinder with the samples separated from adjacent samples by means of a polyurethane disk. This configuration was designed to approximate the spacing and geometry of L1 through L4 vertebrae. The bone samples were then secured in place with nylon screws. The polycarbonate, polyurethane and nylon materials were chosen since they recorded negligent distortion of the DEXA scanners. BMC (g), projected area (cm^2), and ABMD (g/cm^2) values were measured for each of the bone samples by a DEXA (Hologic QDR-1000) scanner. The LBMD was also determined for each sample by dividing the BMC by the segment length as measured with calipers.

After the DEXA scans were performed, each sample was dried at 65 ° C for 15.5 days. Drying was deemed sufficient when the average observed change in mass per day was less than 1 %. The dried sample mass was recorded, and the AWR (g/g) was then calculated as the BMC divided by the dried sample mass. Next, the volume of each sample was determined by means of water displacement as measured with a precision ruled (+/- 0.05 cm^3) graduated cylinder. The VBMD (g/cm^3) was found as the BMC divided by the measured sample volume. For both studies, linear regression analysis was used to compare LBMD, ABMD, and VBMD to the AWR. It was assumed that AWR was the true measure of bone mineralization. Linear regression analysis was also used to examine the effects of subject stature, weight, and age.

Multiple Subject Samples

To compare DEXA measured values for upper extremity long bones, samples from multiple subjects were evaluated. This study utilized 35 mid-shaft samples from 12 female cadavers (Age 50 ± 8 years, 161 ± 7 cm, 62 ± 8 kg). The 35 samples were comprised of mid-shaft segments of 8 humeri, 13 radii, and 14 ulnae as shown in Figure 1. The LBMD, ABMD, VBMD, and AWR were determined for each sample.
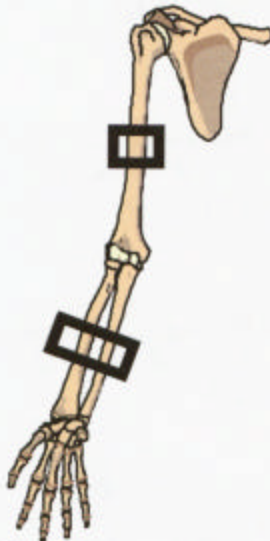

Figure 1. Samples for the Multiple Subject Study Were Taken from the Mid-shaft Humerus, Radius, and Ulna.

Single Subject Samples

In order to remove subject variance such as stature, weight, and age, samples from a single bone from an individual cadaver were evaluated. For the humerus, 9 samples were taken from one female (51 years, 52 kg, 160 cm). The samples were approximately 3 cm in length and comprised the entire humerus. A second female subject (41 years, 56 kg, and 171 cm) was used for samples of the radius and ulna. The radius was divided into 8 segments while the ulna was divided into 7 segments. The samples were taken as shown in Figure 2. As with the humerus, the entire radius and ulna was used. The LBMD, ABMD, VBMD, and AWR were determined for each sample.
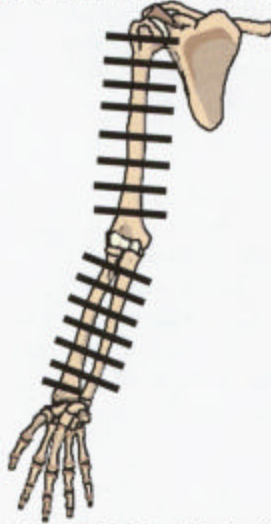

Figure 2. Samples for the Single Subject Study Were Comprised the Entire Humerus, Radius, and Ulna.

RESULTS

Multiple Subject Samples

Table 1 presents the BMC, AWR, LBMD, ABMD, VBMD, and the anthropometric details of each sample from the multiple subject study. Also, the average and standard deviation for each bone group is presented. Overall, the average AWR of each bone group fell between the range of 0.5 g/g and 0.7 g/g as suggested by Cowin et al. (1989) [31]. While it is difficult to find other direct comparisons, Sievanene et al. (1993) found an average ABMD of male mid-shaft radii to be 0.790 ± 0.073 g/cm^2, which is within one standard deviation of the presented average radius ABMD of 0.707 ± 0.126 g/cm^2. For the ulna, Jurist et al. (1977) found an average ABMD of male and female mid-shaft ulnae to be 0.723 ± 0.134 g/cm^2, which is very similar to the average ulna ABMD of 0.707 ± 0.126 g/cm^2 as presented in Table 1 [5]. These close comparisons suggest a validation of the procedures adopted in this study.

For the multiple subject samples, linear regression analysis revealed no correlation between AWR and subject age ($R^2 = 0.0003$), weight ($R^2 = 0.0058$), or height ($R^2 = 0.0018$). The lack of correlation between AWR and subject anthropometry seems justifiable given the similar characteristics and of the all female sample population. In addition, a better correlation between AWR

and VBMD (R2 = 0.66) was observed compared to the correlation with LBMD (R2 = 0.21) or ABMD (R3 = 0.29) for all samples. Better correlations were observed when the samples were separated by each bone group as shown in Table 2. The linear regression analysis revealed that the AWR correlated to VBMD much better than the LBMD and ABMD for all three bone groups.

Table 1. Cadaver Subject Details and DEXA Results from the Multiple Subject Study.

Sample Number		Age	Height (cm)	Weight (kg)	Bone Mineral Content (g)	Ash Weight Ratio (BMC/Dry Weight)	BMC/Length	ABMD (g/cm2)	Measured VBMD (g/cm3)
Humerus	1	61	160	52.2	2.320	0.537	0.773	0.509	0.829
	2	45	161	74.8	2.310	0.464	0.770	0.481	0.642
	3	45	154	64.9	4.980	0.594	1.660	0.901	0.996
	4	45	154	64.9	4.910	0.589	1.637	0.882	1.002
	5	41	171	56.2	5.640	0.602	1.880	0.931	1.025
	6	41	171	56.2	5.890	0.620	1.963	0.939	1.033
	7	45	153	71.7	5.680	0.638	1.893	1.040	1.136
	8	54	153	71.7	5.600	0.591	1.867	1.041	1.057
Average		47	160	64.1	4.666	0.579	1.555	0.840	0.965
Std Dev		7	8	8.4	1.491	0.055	0.497	0.221	0.157
Radius	9	45	161	74.8	1.280	0.570	0.427	0.438	0.800
	10	45	161	74.8	1.380	0.590	0.460	0.450	1.150
	11	59	154	64.9	2.320	0.538	0.773	0.697	0.829
	12	59	154	64.9	2.400	0.564	0.800	0.745	1.091
	13	41	171	56.2	2.510	0.578	0.837	0.763	1.046
	14	41	171	56.2	2.570	0.571	0.857	0.760	1.028
	15	54	153	71.7	2.450	0.595	0.817	0.768	1.225
	16	66	161	59.4	3.040	0.570	1.013	0.798	0.981
	17	66	161	59.4	3.180	0.576	1.060	0.828	1.060
	18	57	167	53.1	2.420	0.551	0.807	0.651	0.864
	19	57	167	53.1	2.750	0.595	0.917	0.718	1.019
	20	50	159	49.4	2.680	0.606	0.893	0.791	0.957
	21	50	159	49.4	2.610	0.593	0.870	0.791	1.044
Average		53	161	60.6	2.430	0.577	0.810	0.707	1.007
Std Dev		9	6	9.0	0.547	0.019	0.182	0.126	0.122
Ulna	22	45	161	74.8	1.490	0.563	0.497	0.456	0.993
	23	45	161	74.8	1.270	0.526	0.423	0.447	0.747
	24	59	154	64.9	3.370	0.640	1.123	0.736	1.162
	25	59	154	64.9	2.480	0.548	0.827	0.736	0.919
	26	41	171	56.2	2.710	0.573	0.903	0.753	1.084
	27	41	171	56.2	2.660	0.592	0.887	0.769	0.950
	28	54	153	71.7	2.770	0.574	0.923	0.761	1.065
	29	54	153	71.7	2.570	0.554	0.857	0.726	0.918
	30	66	161	59.4	3.350	0.673	1.117	0.770	1.196
	31	66	161	59.4	3.150	0.599	1.050	0.840	1.050
	32	57	167	53.1	2.610	0.535	0.870	0.786	0.900
	33	57	167	53.1	2.970	0.538	0.990	0.746	0.900
	34	50	159	49.4	2.530	0.587	0.843	0.816	1.150
	35	50	159	49.4	3.080	0.617	1.027	0.868	1.141
Average		53	161	61.4	2.644	0.580	0.881	0.729	1.013
Std Dev		8	6	9.1	0.612	0.042	0.204	0.125	0.130

Table 2. Linear Regression Correlation Coefficients for the Multiple Subject Study

Bone	LBMD	ABMD	VBMD
Humerus	0.82	0.81	0.96
Radius	0.01	0.02	0.37
Ulna	0.44	0.18	0.74

Single Subject Samples

The BMC, AWR, LBMD, ABMD, and VBMD of each sample for the humerus, radius, and ulna are presented in Table 3. Given that the samples for this study were taken throughout the entire length of the individual bone, the standard deviations for the AWR were higher for the single subject samples at 0.073 g/g, 0.088 g/g, and 0.054 g/g compared to the multiple subject samples at 0.055 g/g, 0.019 g/g, 0.042 g/g for the humerus, radius, and ulna respectively.

Table 3. DEXA Results from the Single Subject Study.

Sample Number	Bone Mineral Content (g)	Ash Weight Ratio (BMC/Dry Weight)	BMC/Length	ABMD (g/cm2)	Measured VBMD (g/cm3)
Humerus 36	7.010	0.386	4.673	0.443	0.539
37	2.330	0.534	0.777	0.441	0.647
38	2.540	0.545	0.847	0.480	0.794
39	2.250	0.531	0.750	0.484	0.776
40	2.320	0.537	0.773	0.509	0.829
41	2.400	0.545	0.800	0.513	0.889
42	2.230	0.597	0.743	0.509	1.014
43	2.350	0.519	0.783	0.571	0.783
44	4.520	0.384	1.507	0.653	0.452
Average	3.106	0.509	1.295	0.511	0.747
Std Dev	1.634	0.073	1.290	0.066	0.174
Radius 45	2.640	0.366	0.880	0.558	0.362
46	2.270	0.515	0.757	0.678	0.873
47	2.380	0.571	0.793	0.730	1.190
48	2.510	0.578	0.837	0.763	1.046
49	2.800	0.596	0.933	0.761	1.077
50	2.260	0.600	0.753	0.668	1.130
51	1.990	0.564	0.663	0.569	0.948
52	1.590	0.416	0.530	0.464	0.548
Average	2.305	0.526	0.768	0.649	0.897
Std Dev	0.381	0.088	0.127	0.108	0.294
Ulna 53	6.380	0.476	1.595	0.629	0.423
54	3.970	0.592	1.323	0.794	0.993
55	3.050	0.612	1.017	0.738	1.089
56	2.710	0.573	0.903	0.751	1.084
57	2.220	0.592	0.740	0.760	1.110
58	2.180	0.591	0.727	0.630	1.038
59	2.020	0.492	0.505	0.451	0.532
Average	3.219	0.561	0.973	0.679	0.895
Std Dev	1.547	0.054	0.376	0.119	0.290

A linear regression analysis again found a better correlation between AWR and VBMD compared to LBMD and ABMD as seen in Table 4. The correlation coefficients for LBMC and ABMD ranged between 0.06 and 0.60, while the VBMD correlation coefficients were much higher and ranged between 0.84 and 0.95.

Table 4. Linear Regression Correlation Coefficients for the Singe Subject Study

Bone	LBMD	ABMD	VBMD
Humerus	0.56	0.10	0.84
Radius	0.06	0.60	0.95
Ulna	0.04	0.54	0.94

To examine the VBMD throughout the length of the bone, the VBMD for each sample was plotted as a function of distance from the proximal end as shown in Figure 3. A similar trend for each bone was observed as the BMC per unit volume of bone decreases at both ends of the humerus, radius, and ulna. This decrease in VBMD corresponds to the increase in proportion of cancellous versus cortical bone in the ends of the bones. As the ratio of cancellous to cortical bone increases, the porous cancellous bone increases the volume and thus, the VBMD decreases. In addition, bone marrow was present in the porous metaphyseal samples which also increased the measured volume and decrease the VBMD.
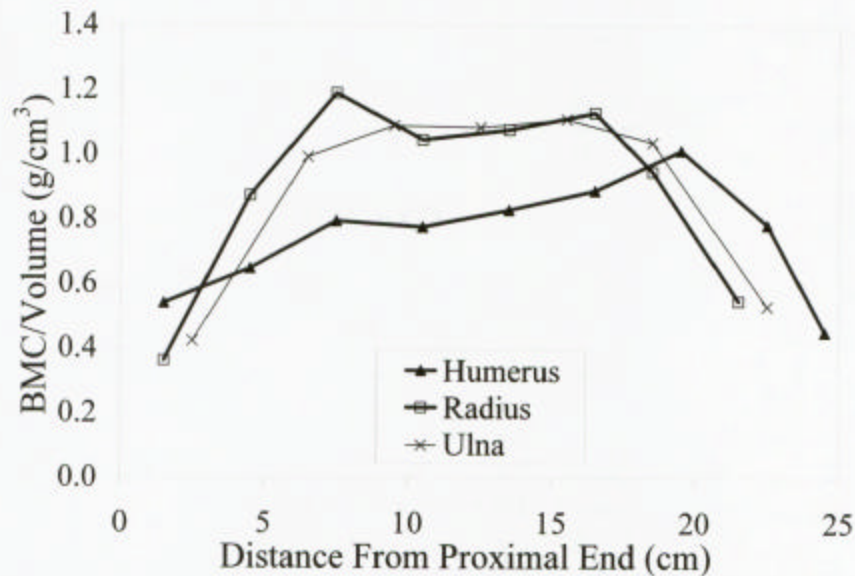

Figure 3. VBMD as a Function of Bone Length as Measured from the Proximal End.

DISCUSSION

The data from both studies suggest that VBMD correlates best with AWR. The VBMD is a relative measurement that does not rely on the projected area of the scan, which can be misleading. For example, a perfect rectangle measuring 1 cm by 4 cm by 4 cm could have a projected area of 4 cm^2 or 16 cm^2 depending on the orientation. When determining the ABMD of a single specimen, the measured BMC will not change with changes in specimen orientation, but these changes in orientation may have a large affect on the resulting ABMD. The possible variations in ABMD and the strong correlation between VBMD and AWR suggest that VBMD or AWR should be used to identify the mineralization of a cadaveric bone specimen.

The primary goal of measuring the level of mineralization in the cadaveric specimen is to gain insight into the relative strength of that bone specimen compared to other subjects. The AWR describes the amount of mineral per unit mass of bone, which typically does not change even with osteoporosis. In patients suffering from osteoporosis, the bone has the same level of mineralization, but there is just less bone as it becomes porous. This porosity directly affects the bone's strength. Thus, VBMD would be the best indicator of the bone's level of porosity, or strength, given that VBMD accounts for the increase in porosity by recording larger volumes and thus lower VBMD for the same BMC. The LBMD and ABMD would not show the change in porosity in this manner.

While VBMD is suggested as the best representation of bone mineralization due to its correlation with AWR and ability to indicate the level of porosity in the cadaveric specimen, it is important to note that the BMC and ABMC as presented directly from a DEXA scan have advantages when examining living subjects. The advantage lies primarily in the large number of similar scans that may be used for comparison. Additionally, DEXA scans in the human are performed in a very similar fashion each time. For example, DEXA scans of the lumbar vertebrae are commonly reported in ABMC and performed in the identical test set up between subjects. Thus, the large data base of similar scans allows for the comparison of a patient's ABMC to other patient's scans as a relative measure or in order to identify possible pathology.

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REFERENCES

Martin RB, Ishida J 1989 The relative effects of collagen fiber orientation, porosity, density, and mineralization on bone strength. J Biomechanics 22:419-426.

Mazess RB, Barden H, Ettinger M 1989 Advances in noninvasive bone measurement. Ann Biomed Eng 17:177-181.

Johnston CC, Slemenda CW, Melton LJ 1991 Clinical use of bone densitometry. New Engl J Med 324:1105-1109.

Black DM, Cummings SR, Genant HK, Nevitt MC, Palermo L, Browner W 1992 Axial and appendicular bone density predict fractures in older women. J Bone Miner Res 7: 633-638.

Jurist JM, Foltz AS 1977 Human ulnar bending stiffness, mineral content, geometry and strength. J Biomechanics **10**:455-459.

Lu PW, Cowell CT, Lloyd-Jones SA, Briody JN, Howman-Giles R 1996 Volumetric bone mineral density in normal subjects, aged 5-27 years. J Clin Endocrinol Metab **84**:1586-1590.

Ruegsegger P, Durand EP, Dambacher MA 1991 Differential effects of aging and disease on trabecular and compact bone density of the radius. Bone **12**:99-105.

Beck TY, Ruff CB, Scott WW Jr, Plato CC, Tobin JD, Quan CA 1992 Sex differences in geometry of the femoral neck with aging: A structural analysis of bone mineral data. Calcif Tissue Int **50**:24-29.

Smith DM, Khairi MRA, Johnston C, Jr 1975 The loss of bone mineral with aging and its relationship to risk of fracture. J clin Invest **56**:311-318.

Cornell C 1990 Management of fractures in patients with osteoporosis. Orthop Clin North Am **21**:125-141.

Ross PD, Wasnich RD, Vogel JM 1988 Detection of prefracture spinal osteoporosis using bone mineral absorptiometry. J Bone Miner Res **3**:1-11.

Mazess RB, Barden H, Ettinger M, Schultz E 1988 Bone density of the radius, spine, and proximal femur in osteoporosis. J Bone Miner Res **3**:13-18.

Riggs BL, Melton LJ 1986 Involutional osteoporosis. N Engl J Med **314**:1676-1686.

Slovik DM, Tosenthal DI, Doppelt SH, Potts JT, Jr, Daly MA, Campbell JA, Neer RM 1986 Restoration of spinal bone in osteoporotic men by treatment with human parathyroid hormone (1-34) and 1,25-dihydoxybitamin D. J Bone Miner Res **1**:377-381.

Smith EL, Gilligan C, Smith PE, Sempos CT 1989 Calcium supplementation and bone loss in middle-aged women. Am j Clin Nutr **50**:833-842.

Hui SL, Longcope C, Johnston LJ 1987 Sex steroids and bone mass. A study of changes about the time of menopause. J Clin Invest **80**:1261-1269.

Storm T, Thamsborg G, Steiniche T, Genant HK, Sorensen OH 1990 Effect of intermittent cyclical etidronate therapy on bone mass and fracture rate in women with postmenopausal osteoporosis. N Engl J Med **322**:1265-1271.

Riggs BL, Hodgson SF, O'Fallon M, Chao EYS, Wahner HW, Mubs JM, Cedel SL, Melton LJ III 1990 Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med **322**:802-809.

Watts NB, Harris ST, Genant HK, Wasnich RD, Miller PD, Jackson RD, Licata AA, Ross P, Woodson GC 3rd, Yanover MS, et al. 1990 Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. N Engl J Med **323**:73-79.

Cann CD 1988 Quantitative CT for determination of bone mineral density: a review. Radiology **166**:509-522.

Kalender WA 1992 Effective dose values in bone mineral measurement by photon absorptiometry and computed tomography. Osteoporosis Int **2**:82-87.

Stein JA, Lazewatsky JL, Hochberg AM 1987 Dual-energy x-ray bone densitometer incorporating an internal reference system. Radiology **165** (suppl):313.

Sorenson JA, Duke PR, Smith SQ 1989 Simulation studies of dual energy s-ray absorptiometry. Med Phys **16**:75-80.

Lewis MK, Blake GM, Fogelman I 994 Patient dose in dual x-ray absorptiometry. Osteoporosis Int **4**:11-15.

Blake GM, Tong CM, Fogelman I 1991 Intersite comparison of Hologic QDR-1000 dual energy x-ray bone densitometer. Br J Radiol **64**:440-446.

Sabin MA, Blake GM, MacLaughlin-Black SM, Fogelman I 1995 The accuracy of volumetric bone density measurements in dual x-ray absorptiometry. Calcif Tissue Int **56**:210-214.

Sievanene H, Kannus P, Oja P, Vuori I 1993 Precision of dual energy x-ray absorptiometry in the upper extremities. Bone and Mineral **20**:235-243.

Larcos G, Wahner HW 1991 An evaluation of forearm bone mineral measurement with dual-energy x-ray absorptiometry. J Nucl Med **32**:2101-2106.

Sievanen H, Kannus P, Oja P, Vuori I 1994 Dual energy x-ray absorptiometry is also an accurate and precise method to measure the dimensions of human long bones. Calcif Tissue Int **54**:101-105.

Hsu ES, Patwardhan AG, Meade KP, Light TR, Martin WR 1993 Cross-sectional geometrical properties and bone mineral contents of the human radius and ulna. J Biomechanics **26**:1307-1318.

Cowin S, VanBuskrik W, Ashman R 1987 Properties of Bone. In Handbook of Bioengineering by Skalak R, and Chien S, McGraw Hill, New York.